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Lactobacillus casei ATCC 393 combined with vasoactive intestinal peptide alleviates dextran sodium sulfate-induced ulcerative colitis in C57BL/6 mice *via* NF-κB and Nrf2 signaling pathways

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ABSTRACT

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) which is related to an immunological imbalance of the intestinal mucosa. Many clinical evidences indicate probiotics supplementation appears to be effective and safe in patients with UC. Vasoactive intestinal peptide (VIP) is an endogenous neuropeptide with multiple physiological and pathological effects. In this study, we investigated the protective effect of the combination of *Lactobacillus casei* ATCC 393 (*L. casei* ATCC 393) with VIP on dextran sodium sulfate (DSS)-induced UC in mice and the potential mechanism. The results showed that, compared with the control group, DSS treatment significantly shortened the colon length, caused inflammation and oxidative stress, and further resulted in the intestinal barrier dysfunction and gut microbiota dysbiosis. In addition, intervention with *L. casei* ATCC 393, VIP or *L. casei* ATCC 393 combined with VIP significantly reduced UC disease activity index. However, compared with *L. casei* ATCC 393 or VIP, *L. casei* ATCC 393 combined with VIP effectively relieved symptoms of UC by regulating immune response, enhancing antioxidant capacity, and regulating nuclear factor kappa-B (NF-κB) and nuclear factor erythroid-derived-2-like 2 (Nrf2) signaling pathways. In conclusion, this study suggests that *L. casei* ATCC 393 combined with VIP can effectively relieve DSS-induced UC, which is a promising treatment strategy for UC.

1. Introduction

Ulcerative colitis (UC), with the clinical symptoms of diarrhea, abdominal pain, weight loss and even bloody stools, is a kind of inflammatory bowel disease (IBD) [1]. The pathogenesis of UC is multifactorial, involving genetics, environmental factors, microbiota and the immune system [2]. With the increasing incidence of UC in the world, prevention and treatment of UC has attracted more and more attention. Recent studies have shown that the function and application value of glycans and glycosylation has gradually emerged in gastrointestinal inflammation. Inverting glycosyltransferase catalyzes the glycosylation modification of arginine, which can effectively alleviate intestinal diseases caused by enteropathogenic *Escherichia coli* (EPEC) infection [3]. Currently, the main clinical drugs for UC include 5-aminosalicylic acid agents, glucocorticoids, antibiotics, immunomodulators and steroid hormones [4]. However, these drugs only alleviate some symptoms of UC and have obvious side effects [5]. Therefore, it is vital importance to develop an effective treatment strategy with few side effects for UC. Numerous studies have shown that inflammation, oxidative stress, and gut microbiota dysbiosis usually occur in patients with UC [6,7]. Therefore, inhibition of UC-associated pathophysiology, including oxidative stress, inflammation and gut microbiota dysbiosis, is a promising strategic direction to delay UC progression.

Consuming probiotics may help restore the balance of gut microbiota, enhance intestinal mucosal barrier function, and reduce gastrointestinal infections [8]. Several studies have shown probiotics are as effective as traditional drug therapy used in treatment of UC. A clinical trial involving 1763 patients showed that probiotics significantly improved clinical remission rates in patients with UC [9]. Dietary supplement with *Lactobacillus fermentum* in UC patients can inhibit the activation of nuclear factor kappa-B (NF-κB), thereby reducing interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) levels and have the effect of alleviating UC [10]. It was worth noting that *Bifidobacterium breve* alleviated UC by reducing shedding of apoptotic epithelial cells in an extracellular polysaccharide and myeloid differentiation factor 88 (MyD88)-dependent manner [11]. Additionally,

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clinical study showed that the addition of *Lactobacillus* species to conventional treatment reduces relapse in UC patients [12]. A study found dairy-derived *Lactobacillus delbrueckii* modulated the NF-κB pathway and reduced inflammation in dextran sodium sulfate (DSS)-induced UC mice [13]. Lactic acid bacteria, especially the species of the genus *Lactobacillus*, have attracted considerable attention due to their immunomodulatory, anti-inflammatory and anti-cancer activities. Our previous research indicated that *Lactobacillus casei* ATCC 393 (*L. casei* ATCC 393) inhibited enterotoxigenic *Escherichia coli* (ETEC) K88 induced mast cell activation through Toll-Like Recepters (TLRs) signaling pathway, and further alleviates intestinal barrier dysfunction [14]. Furthermore, recent studies showed that oxidative stress can play a crucial role in the occurrence and development of colitis [15]. Excessive production of free radicals can lead to intestinal damage and inflammation-related gut diseases, such as IBD, irritable bowel syndrome (IBS). *Lactobacillus* can scavenge free radicals and has antioxidant effect [16]. It is reported that the addition of *Lactobacillus paracasei* could enhance the antioxidant capacity by increasing the activities of catalase (CAT) and superoxide dismutase (SOD), to achieve the purpose of alleviating UC [17].

In recent years, many studies showed that gut microbiota disorder was closely related to the occurrence of UC. Gut microbiota participates in and influences various physiological activities of human body, especially metabolism and immunity. Among of them, immunoglobulin A (IgA), as the most important antibody in intestinal mucosa, wraps microorganisms, which is the sign of gut microbiota activating immune response, and the absence of secretory IgA (sIgA) will also increase the risk of UC [18]. Many studies showed that the decline of diversity and richness of gut microbiota was related to the increase of the prevalence of colitis. In UC patients, the ratio of Firmicutes to Bacteroides (F/B) decreased, while the abundance of *Proteobacteria* increased significantly [19]. In addition, the disturbance of gut microbiota can lead to dysfunction of intestinal epithelial cells and injury of intestinal mucosa, and finally increase the permeability of intestinal barrier. The research reported that *Lactobacillus casei* can improve intestinal integrity by regulating gut microbiota, thereby reducing intestinal mucosa damage [20]. Therefore, regulating the composition of gut microbiota by supplementing probiotics may be an effective measure for the treatment of UC.

Vasoactive intestinal peptide (VIP), a neuropeptide that functions as a neuromodulator and neurotransitter, is considered as a potential drug candidate for the treatment of inflammatory and autoimmune diseases, including acute pancreatitis, infectious shock, IBD, lipopolysaccharide-induced acute inflammation and arthritis [21,22]. It is reported that daily injection of 1 or 5 nmol of VIP in mice was effective in relieving 2, 4,6-trinitrobenzenesulfonic acid (TNBS)-induced Crohn's disease with a significant reduction in histopathological severity [23]. It has also been found that VIP can regulate the expression of interleukin-10 (IL-10) in regulatory B cells of patients with colitis, and inhibit autoimmune diseases or excessive inflammation by inhibiting activated macrophages and helper T (Th) cells. Noteworthy, the study also proved that VIP can achieve anti-inflammatory and anti-diarrhea effects through single administration [24]. Moreover, VIP has free radical scavenging properties that can reverse colonic mitochondrial dysfunction in UC, reverse the clinical and histopathological severity of UC, and preserve the integrity of the colonic mucosa [25]. A study found that VIP partially reduced oxidative stress in pancreatic acini by inhibiting NADPH oxidase activity, thereby reducing oxidative stress-induced damage [26]. These studies showed that VIP had a certain antioxidant capacity. Additionally, the research demonstrated that VIP played an indispensable role in regulating colon mucosal integrity and intestinal epithelial barrier homeostasis, and the absence of VIP in proper environment increases the susceptibility of UC [27].

Therefore, this study was conducted to investigate the protective effect of *L. casei* ATCC 393 combined with VIP on DSS-induced UC in mice and its relationship with inflammation, oxidative stress and gut microbiota. In addition, a heatmap of Spearman correlation between the altered gut microbiota and UC related biochemical indexes was generated to identify the potential correlation between gut microbiota and UC.

2. Materials and methods

2.1. Reagents

L. casei ATCC 393 was purchased from Chengdu Chuanshi Kewei Biotechnology Co., Ltd. DSS was purchased from MP Biomedicals (Santa Ana, CA, USA). ELISA kits for assaying interleukin-1β (IL-1β), interleukin-18 (IL-18), IL-10, transforming growth factor beta (TGF-β), TNF-α, myeloperoxidase (MPO) and sIgA were purchased from Jianglai Biological Technology Co., Ltd (Shanghai, China). Primary antibodies against occludin, claudin1, β-actin, nuclear factor (erythroid-derived-2)-like 2 (Nrf2), heme oxygenase 1 (HO-1), NADH quinone oxidoreductase 1 (NQO1), toll-like receptor 4 (TLR4), IL-1β, MyD88, I-kappa-B kinase alpha (IKKα), NF-kappa-B inhibitor alpha (IKBα), Phospho-IKBα (p-IKBα), NF-κB, Phospho-NF-κB (p-NF-κB) and horseradish peroxidase (HRP)-labelled secondary antibody were procured from ABclonal Biotechnology (Wuhan, China). RIPA protein lysate, PMSF protease inhibitor, BAC protein concentration detection kit, Total superoxide dismutase (T-SOD), malondialdehyde (MDA), and glutathione peroxidase (GSH-Px) were purchased from Solarbio Technology Co., Ltd (Beijing, China). The PVDF membrane was purchased from Millipore, USA, the ECL chemiluminescence kit was purchased from Bio-Rad, USA.

2.2. Culture of L. casei ATCC 393

L. casei ATCC 393 was streaked and cultured into MRS solid medium to obtain a single colony. Then, a single colony was inoculated into MRS broth medium and cultured to $OD_{600} = 2.0$ as seed solution of *L. casei* ATCC 393. Finally, the seed solution was inoculated into MRS medium at 1/1000 inoculum and cultured for 12 h to a concentration of 1×10^9 CFU/mL. The obtained *L. casei* ATCC 393 fermentation broth was used for the following experiment.

2.3. Animal experimental design

The animal experiment was approved by the Institutional Animal Care and Use Committee of the Northwestern Polytechnical University (Permit Number: 202201097), and the study was conducted in strict accordance with the guidelines of the National Institutes of Health guidelines for the care and use of experimental animals. The animal experimental scheme was shown in Fig. 1A. Briefly, fifty males C57BL/6 mice (body weight: 20 ± 1 g) were purchased from the Huafukang Biological Technology Co., Ltd (Beijing, China). During the whole experimental period, the mice were fed in the Animal Experimental Center of Northwestern Polytechnical University in a room with a temperature of 25 ± 2 °C, relative humidity of 50 ± 5 %, and a 12 h light/dark cycle. After an adaptive period of 7 days, the mice were randomly assigned to five groups with 10 mice per group: control group, DSS model group, *L. casei* ATCC 393 + DSS group, VIP + DSS group, and *L. casei* ATCC 393 + VIP + DSS group. The mice in the *L. casei* ATCC 393 + DSS group and *L. casei* ATCC 393 + VIP + DSS group were orally administered with 200 μL of *L. casei* ATCC 393 fermentation broth containing 1×10^9 CFU/mL daily for 4 weeks. Other experimental mice were given 200 μL of fresh MRS medium per day by oral gavage. On day 29 of the experiment, the mice in the DSS-treatment groups, *L. casei* ATCC 393 + DSS groups, VIP + DSS groups and *L. casei* ATCC 393 + VIP + DSS groups were drinked water ad libitum containing 3 % DSS (w/v, at a molecular weight of 36,000–50,000) for 7 days. The mice in the VIP + DSS group and *L. casei* ATCC 393 + VIP + DSS group were given 100 μL of 0.25 nmol VIP by intraperitoneal injection on day 1, 3, 5 of the consecutive 7 days. For the duration of the experiment, the status, weight and food intake of mice were monitored and recorded every day.

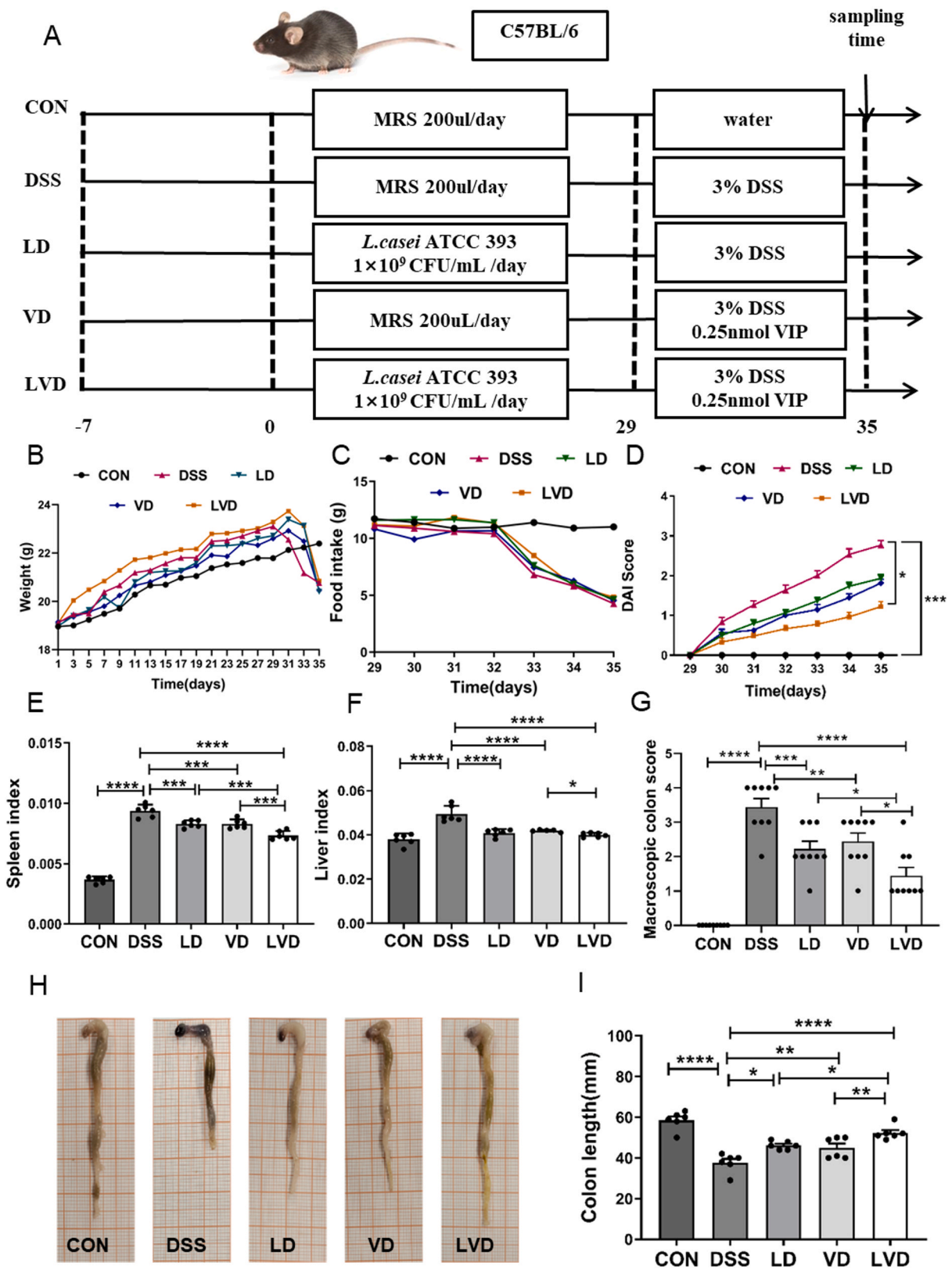

Fig. 1. The protective effect of *L. casei* ATCC 393 combined with VIP on UC mice induced by DSS. (A) The experimental scheme; (B) The average weight of mice during the experiment period; (C) The food intake of mice during the seven-day modeling period; (D) The DAI in mice during the seven-day modeling period; (E) The spleen index of mice; (F) The liver index of mice; (G) Blinded histologic scoring of colons; (H) The colon length of mice; (I) Quantitative analysis of colon length before and after treatment with *L. casei* ATCC 393 and VIP. All data are presented as the mean ± S.E.M (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ (CON: the control group; LD: *L. casei* ATCC 393 + Dextran sodium sulfate; VD: Vasoactive intestinal peptides+ Dextran sodium sulfate; LVD: *L. casei* ATCC 393 + Vasoactive intestinal peptides+ Dextran sodium sulfate).

2.4. Disease activity index

The severity of UC was assessed by disease activity index (DAI). During the experiment, the mice were monitored and recorded daily for weight, viscosity of stool and blood in their stool. The DAI was calculated according to the following formula: DAI = (score of stool viscosity + score of degree of blood in stool + percentage of weight loss)/3 [28,29]. The specific scoring standards are shown in Supplementary Table S1.

2.5. Samples collection

After the above treatments, the experimental mice were anesthetized with ether and blood was quickly collected from the eyeballs. The serum samples were separated by centrifuging at 3500 rpm for 10 min at 4 °C. Then, lift the abdominal wall with pliers and open the abdominal cavity. The intact colon was separated and placed on a measuring paper, and the length of the colon was recorded. Then, a portion of the colon was stored in 4 % paraformaldehyde for subsequent histological evaluation, while the rest of the colon samples were frozen with liquid nitrogen and then stored at −80 ℃ for subsequent experimental analysis. Colon contents were collected aseptically, frozen in liquid nitrogen and stored at −80 ℃ for subsequent microbiome analysis.

2.6. Measurement of organ index

The mice were anesthetized with ether and cervical dislocation to death. The abdominal cavity was immediately opened, the heart, liver, kidneys, spleen, and lung of the mice were quickly removed. After washing with sterile water, the water was wiped dry. Each organ was weighed and the organ index was calculated by the following formula: Organ index = weight of organ (g) / weight of body (g).

2.7. Routine blood test

After the mice were anesthetized with ether, blood from the eyeballs was collected into a 0.5 mL anticoagulation centrifuge tube. The blood routine analysis was carried out by an automated hematology analyzer (OLYMPUS AU400, Japan).

2.8. Macroscopic and histopathological analysis

0.5 cm of distal colon tissue was taken from anesthetized mice and placed in 4 % paraformaldehyde, then dehydrated with alcohol and made transparent by xylene. Then the transparent colon tissue was immersed in paraffin and embedded, cut into 5 μm, and the sections were stained with H&E. Finally, dehydrate and be transparent again for sealing. The images of tissue sections were obtained by the tissue slicing scanner and analyzed using the native software of the tissue slicing scanner. Then, macroscopic evidence of inflammation was scored using the method described previously [30]. Briefly, the colon has a normal morphology and a score of 0; in the absence of visible hyperemia, mild intestinal wall thickening was rated as 1 point; while moderate intestinal wall thickening and hyperemia were rated as 2 points; severe intestinal wall thickening with stiffness and significant hyperglycemia were rated as 3 points; severe intestinal wall thickening and stiffness, hyperemia, and colon adhesions were rated as 4 points [31].

2.9. In vivo intestinal permeability test

Mice fasted for 4 h before FITC-dextran (Sigma-Aldrich, USA) were administered orally with FITC-dextran 4 kDa (400 mg/kg B.W.). 2 h after dosing, mice were anesthetized using ether, blood was collected from the eyeballs and serum was prepared. Serum from PBS dosed mice were used for the standard curve. FITC-dextran concentrations in serum were analyzed using a Multi-Mode Microplate Reader (Bio-Rad, Hercules, USA) with excitation λ485 nm and emission λ535 nm.

2.10. Determination of inflammatory cytokines levels in colon and serum

The distal colon tissue was placed in a 1.5 mL centrifuge tube with lysate. Then the samples were broken up with an ultrasonic crusher and centrifuged. After centrifugation, the supernatant was collected and the protein concentration of all samples was detected by BCA protein assay kit, and the protein concentration of all samples was adjusted to the same concentration. The levels of IL-1β, IL-18, IL-10, TNF-α and TGF-β in colon and serum were detected by the corresponding kits according to the manufacturer's instructions.

2.11. Determination of sIgA and MPO levels in serum

The levels of sIgA (Cat# JL11763; Jianglaibio) and MPO (Cat# JL10367; Jianglaibio) were detected by ELISA kit according to the corresponding manufacturer's instructions. Briefly, antibodies were coated in each well of a 96-well ELISA plate. Enzyme-labeled antibodies bind to the serum samples to be tested to form an antibody-antigen-enzyme labeled antibody complex. After washing and adding a chromogenic substrate, the optical density (OD) values were detected using a microplate reader (Bio-Rad, Hercules, USA) at a wavelength of 450 nm.

2.12. Evaluation of antioxidant capacity

The preparation method of colonic protein sample was the same as described in 2.10. The activities of T-SOD (Cat# BC0175; Solarbio) and GSH-Px (Cat# BC1195; Solarbio), and the content of MDA (Cat# BC0025; Solarbio) were measured using the commercial assay kits according to the manufacturer's instructions.

2.13. Western blot analysis of NF-κB and Nrf2 signaling pathways

The preparation method of colonic protein sample was the same as described in 2.10. SDS-PAGE gels were loaded with equal volumes and amounts of protein from the samples and subjected to electrophoresis to separate proteins by size. After separation, proteins were transferred to PVDF membrane. The membranes were incubated overnight at 4 °C with the appropriate primary antibodies for claudin 1, occludin, TLR4, MyD88, IKKα, IKBα, p-IKBα, NF-κB, p-NF-κB, IL-1β, Nrf2, HO-1, NQO1 and β-actin followed by incubation for 1 h at room temperature with the appropriate secondary antibodies. After incubation with primary and secondary antibodies, the immunoreactive protein bands were visualized using an ECL Substrate kit and a T5200 Multi Detection System (Tanon, Shanghai, China). Image J (National Institutes of Health (NIH), Bethesda, MD) was used to analyze the gray value of the target protein bands by the expression level of internal reference β-actin.

2.14. 16S rRNA gene sequencing

The 16S rRNA gene amplicon was pyrosequencing using Shenzhen HiSeq platform (BGI Technology Co., LTD., Shenzhen, China) to analyze the bacterial diversity and microbial community composition of colon contents in mice. The PCR reaction system was configured with 30 ng of qualified genomic DNA sample and corresponding fusion primers, and PCR reaction parameters were set for PCR amplification. The PCR amplification products were purified and dissolved in Elution Buffer using Agencourt AMPure XP magnetic beads and labeled to complete library construction. Agilent 2100 Bioanalyzer was used to detect the fragment range and concentration of the library. According to the size of the inserted fragments, the HiSeq platform was selected for sequencing. Then, the information was analyzed and the VennDiagram in OTUs or taxa was drawn using the R package "VennDiagram" version 3.1.1. Species accumulation curves were plotted using R software package version 3.1.1. Core-pan OTUs for more than six samples were plotted

using R package version 3.1.1. The principal component analysis (PCA) in OTUs was plotted with the R package "ade4". OTU Rank curve was plotted with R package version 3.1.1. GraPhlan map of species composition was created using Principal Coordinate Analysis (PCoA) was Performed by QIIME (V1.8.0).

2.15. Statistical analysis

All experimental results were statistically analyzed using GraphPad Prism 8.0 statistical software (Graph-Pad Software, San Diego, CA, USA) and all experimental data were presented as the mean ± standard error of the mean (S.E.M.). The statistical significance was determined by one-way ANOVA. P value < 0.05 was considered as statistically significant difference.

3. Results

3.1. L. casei ATCC 393 combined with VIP alleviates DSS-induced UC in mice

The experimental scheme is shown in Fig. 1A. DSS-induced UC is characterized by significant weight loss, diarrhea, and severe bloody stools. Compared with the control group, the UC resulted in the significant weight loss. However, compared with the DSS model group, administration of *L. casei* ATCC 393, VIP and *L. casei* ATCC 393 combined with VIP effectively alleviated the weight loss of mice exposed to DSS (Fig. 1B). Compared with the control group, the food intake of mice from DSS model group decreased significantly. However, compared with the DSS model group, the food intake of mice in *L. casei* ATCC 393 group, VIP group and *L. casei* ATCC 393 combined with VIP group increased (Fig. 1C). DSS treatment significantly increased the DAI scores of mice compared with the control group. However, the *L. casei* ATCC 393 combined with VIP significantly decreased the DAI scores of mice (Fig. 1D). High spleen index and liver index indicate an inflammatory reaction in the organism, and DSS can cause swelling and heaviness of the spleen and liver. Compared with the control group, DSS exposure significantly increased the spleen index and liver index of mice. Compared with the DSS model group, administration of VIP group and *L. casei* ATCC 393 significantly decreased the spleen index and liver index of mice. Among them, the protective effects of *L. casei* ATCC 393 combined with VIP group on spleen and liver were better than VIP or *L. casei* ATCC 393 (Fig. 1E and F). This indicates that *L. casei* ATCC 393 combined with VIP can effectively alleviate inflammatory response caused by DSS. As shown in Fig. 1G, in the macroscopic score of colitis, compared with the control group, the macroscopic score of colitis in the DSS model group was significantly increased. Compared with the DSS model group, *L. casei* ATCC 393, VIP and the combination of *L. casei* ATCC 393 and VIP all significantly reduced the macroscopic score of colitis. However, the combination of *L. casei* ATCC 393 and VIP showed a better protective effect. In addition, compared with the control group, the colon length of mice from the DSS model group was significantly shortened. Compared with the DSS model group, the colon length of mice from the *L. casei* ATCC 393 group, VIP group and *L. casei* ATCC 393 combined with VIP group were significantly increased. Among them, the colon length of mice from the *L. casei* ATCC 393 combined with VIP group was the longest (Fig. 1H and I).

3.2. Hematological analysis

Blood routine can reflect early signs of many systemic diseases and check the body for infections or inflammatory reactions. As shown in the Fig. 2A-E, compared with the control group, the absolute value of monocytes (MONO#) and platelet count (PLT) in DSS model group were significantly increased, while hemoglobin (HGB), red blood cell count

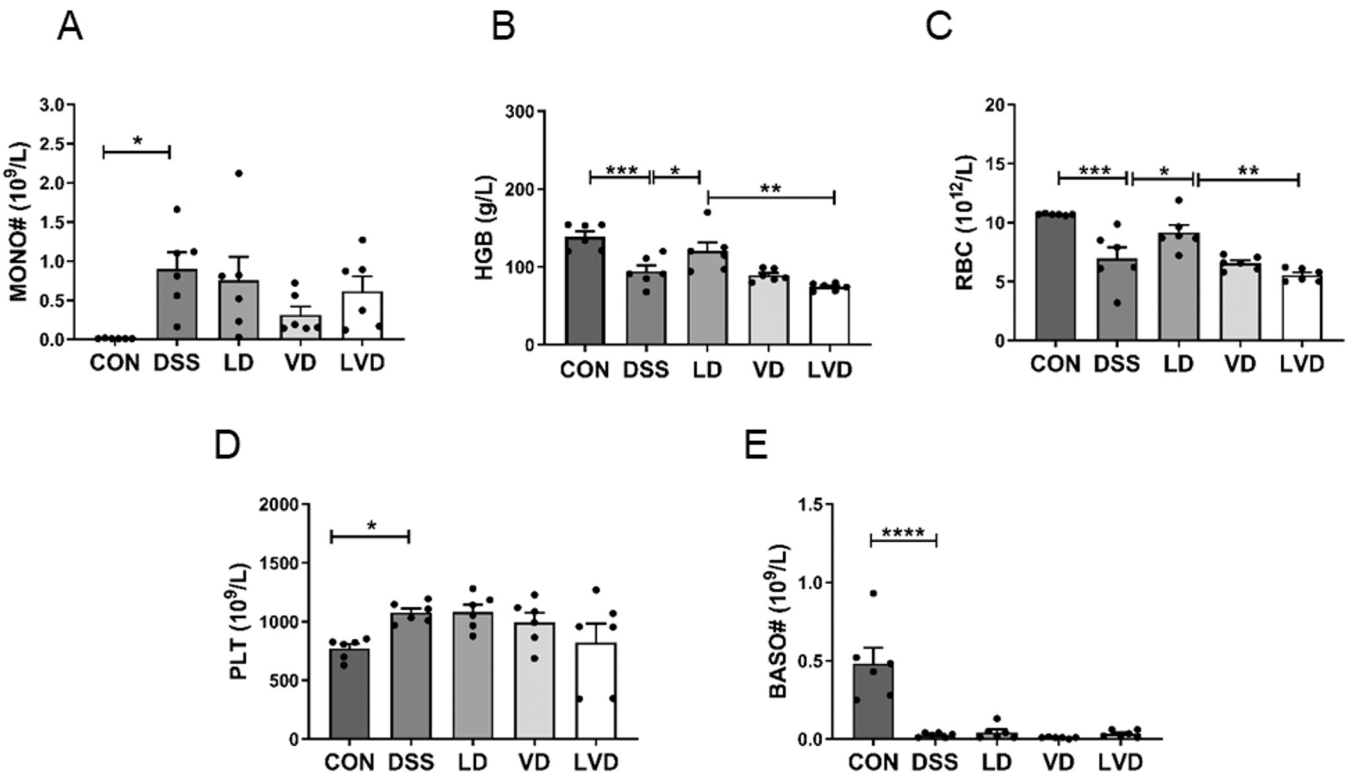

Fig. 2. The blood routine of mice. (A) The level of MONO# in the blood of mice; (B) The level of HGB in the blood of mice; (C) The level of RBC in the blood of mice; (D) The level of PLT in the blood of mice; (E) The level of BASO# in the blood of mice. All data are presented as the mean ± S.E.M (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ (CON: the control group; LD: *L. casei* ATCC 393 + Dextran sodium sulfate; VD: Vasoactive intestinal peptides+ Dextran sodium sulfate; LVD: *L. casei* ATCC 393 + Vasoactive intestinal peptides+ Dextran sodium sulfate).

(RBC) and absolute value of basophils (BASO#) were significantly decreased. Compared with the DSS model group, *L. casei* ATCC 393 combined with VIP group showed a decrease in MONO# and PLT, and an increase in BSAO#. However, there was no significant difference. Blood routine testing is only a basic indicator to measure, and more accurate measurement requires in-depth research, combined with our other experimental results.

3.3. Protective effect of *L. casei* ATCC 393 combined with VIP against DSS-induced intestinal barrier dysfunction in mice

In UC, damage to the intestinal mucosal barrier is the main cause of its occurrence. FITC-dextran, sIgA, H&E and tight junction proteins are important indicators for evaluating intestinal mucosal barrier damage. As shown in Fig. 3A, the level of FITC-dextran in serum in the DSS model group were significantly higher than that in the control group. However, compared with the DSS model group, administration of *L. casei* ATCC 393, VIP and *L. casei* ATCC 393 combined with VIP significantly reduced the levels of FITC-dextran. Among of them, the *L. casei* ATCC 393 combined with VIP group was the most significant. sIgA plays a crucial role in protecting the integrity of the intestinal epithelium. As shown in Fig. 3B, the serum sIgA levels in the DSS model group were significantly lower than that in the control group. However, compared with the DSS model group, *L. casei* ATCC 393, VIP and *L. casei* ATCC 393 combined with VIP significantly increased the level of sIgA. Amony them, the *L. casei* ATCC 393 combined with the VIP group was the most significant. As shown in Fig. 3C, compared with the control group, the mice from DSS model group showed obvious lesions in the distal colonic mucosa, such as colonic mucosal damage, crypt atrophy, degeneration, and intestinal epithelial cell shedding, tissue necrosis, inflammatory edema and a small amount of inflammatory cell leaching (Fig. 3D). However, administration of *L. casei* ATCC 393, VIP and *L. casei* ATCC 393 combined with VIP improved the colon tissue morphology, and *L. casei* ATCC 393 combined with VIP had the most significant improvement effect.

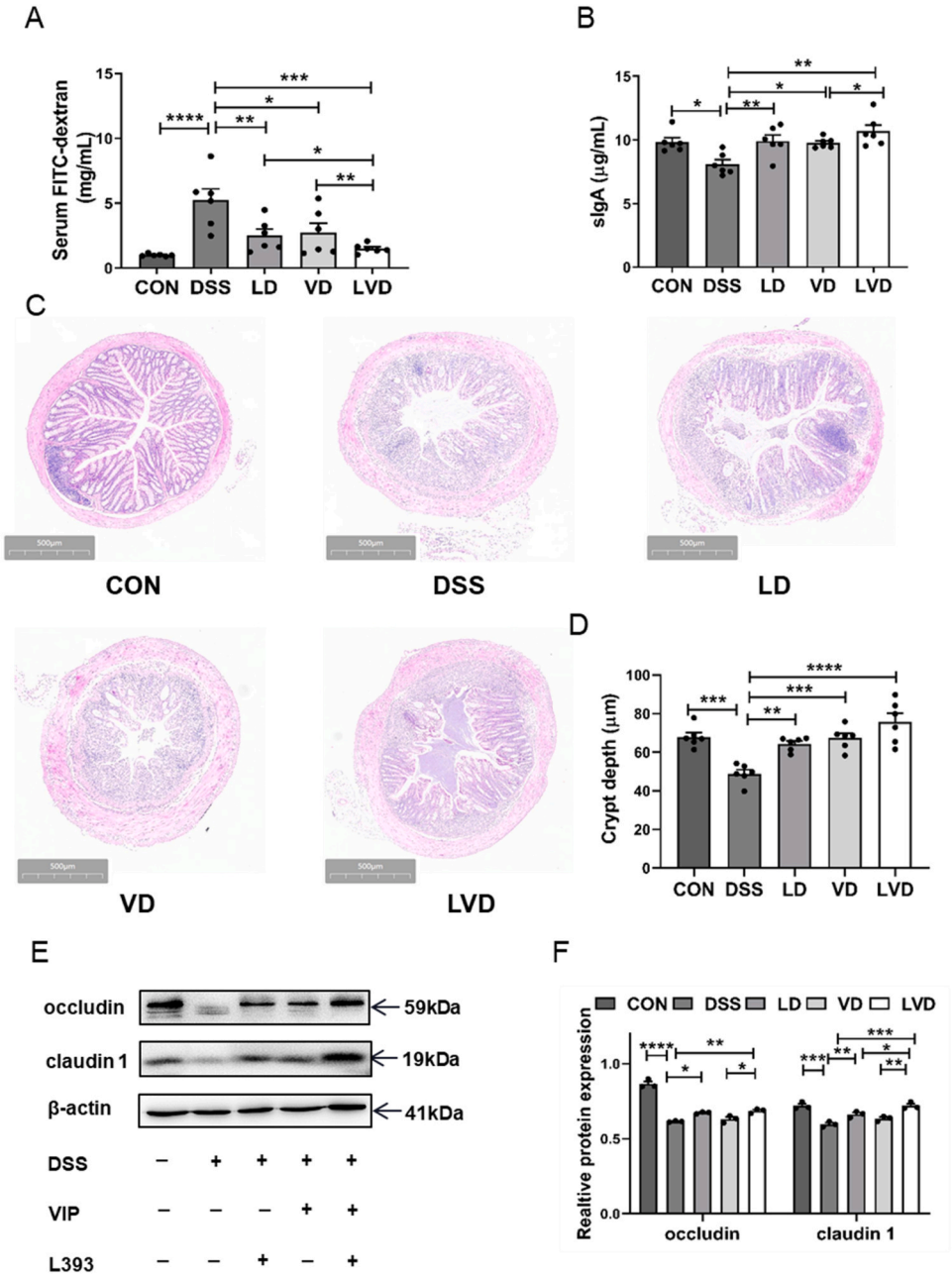

Fig. 3. The protective effect of *L. casei* ATCC 393 combined with VIP on the intestinal barrier function in UC mice induced by DSS. (A) The level of FITC-dextran in the colon of mice; (B) The level of sIgA in the colon of mice; (C) Histological examination of colon tissue stained with H&E (magnification × 500); (D) Quantitative detection of colonic crypt; (E) The expression levels of tight junction proteins (occludin and claudin 1) detected by Western blot analysis; (F) Quantitative analysis of tight junction protein expression level. All data are presented as the mean ± S.E.M (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ (CON: the control group; LD: *L. casei* ATCC 393 + Dextran sodium sulfate; VD: Vasoactive intestinal peptides+ Dextran sodium sulfate; LVD: *L. casei* ATCC 393 + Vasoactive intestinal peptides+ Dextran sodium sulfate).

Compared with the control group, the expression levels of occludin and claudin1 were significantly decreased in mice from the DSS model group. However, compared with the DSS model group, the expression levels of occludin and claudin1 in the colon tissue of mice from the *L. casei* ATCC 393 group, VIP group and *L. casei* ATCC 393 combined with VIP group were significantly increased. Among them, the increase degree was most significant in the *L. casei* ATCC 393 combined VIP group (Fig. 3E and F). These results suggest that *L. casei* ATCC 393 combined with VIP can effectively alleviate DSS-induced intestinal barrier dysfunction.

3.4. *L. casei ATCC 393 combined with VIP enhances the anti-inflammatory ability in mice exposed to DSS*

Further detection of inflammatory response related indicators shows that compared with the control group, the expression levels of pro-inflammatory cytokines TNF-α, IL-1β, and IL-18 in serum and colon of the DSS model group mice were significantly increased, and the anti-inflammatory cytokines TGF-β and IL-10 was significantly decreased (Fig. 4A–E). However, compared with the DSS model group, *L. casei* ATCC 393, VIP and *L. casei* ATCC 393 combined with VIP significantly increased the expression levels of TGF-β and IL-10 in serum and colon, and decreased the levels of TNF-α, IL-1β and IL-18 in serum and colon. Among of them, the levels of TNF-α, IL-1β and IL-18 in serum and colon decreased most significantly in *L. casei* ATCC 393 combined with VIP group, while the levels of TGF-β and IL-10 increased most significantly. MPO is related to both inflammation and oxidative stress. As shown in Fig. 4F, compared with the DSS model group, the content of MPO in the *L. casei* ATCC 393 group, VIP group and *L. casei* ATCC 393 combined with VIP group were significantly decreased. Among them, the *L. casei* ATCC 393 combined with VIP group was the most significantly. These results showed that *L. casei* ATCC 393 combined with VIP group had a strong anti-inflammatory effect on mice exposed to DSS.

3.5. *L. casei ATCC 393 combined with VIP improves the antioxidant capacity of mice exposed to DSS*

Further detection of oxidative stress-related indicators revealed that the activities of T-SOD and GSH-Px in DSS model group were significantly decreased (Fig. 5A and B), and the content of MDA was significantly increased (Fig. 5C). However, compared with the DSS model group, the activities of T-SOD and GSH-Px in *L. casei* ATCC 393 group, VIP group and *L. casei* ATCC 393 combined with VIP group were significantly increased, and the content of MDA was significant decreased. The activity of T-SOD in *L. casei* ATCC 393 group and *L. casei* ATCC 393 combined with VIP group was higher than in the other groups. The activity of GSH-Px in *L. casei* ATCC 393 group was higher than that in the other groups. In addition, the content of MDA in *L. casei* ATCC 393 combined with VIP group was lower than in the other groups. These results suggest that *L. casei* ATCC 393 combined with VIP may have a better antioxidant effect in DSS-induced UC mice.

3.6. *L. casei ATCC 393 combined with VIP inhibits the signal pathway mediated by NF-κB*

To understand the mechanism by which *L. casei* ATCC 393 combined

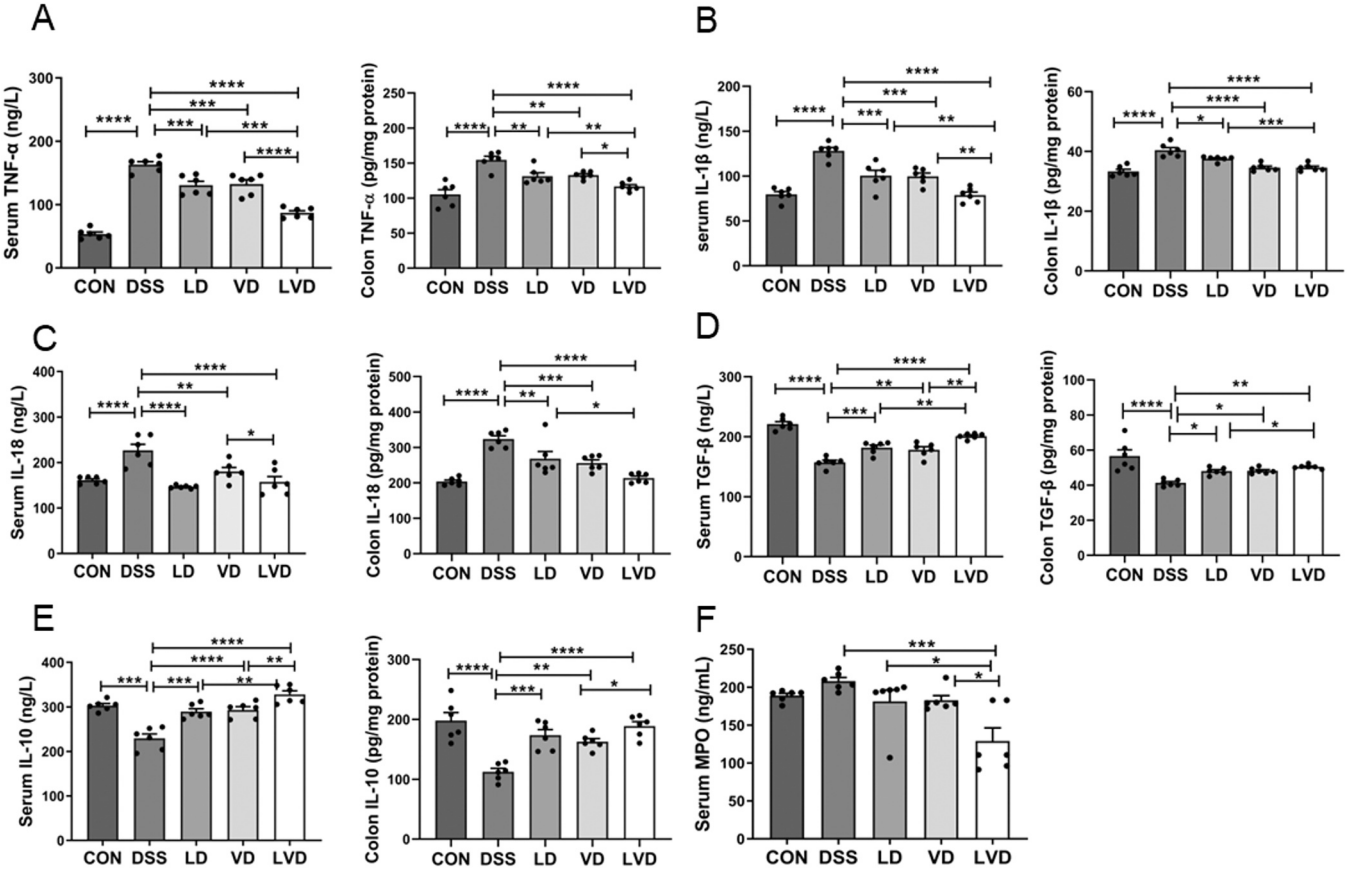

Fig. 4. The effect of *L. casei* ATCC 393 combined with VIP on the inflammatory response in UC mice induced by DSS. (A) The levels of TNF-α in serum and colon; (B) The levels of IL-1β in serum and colon; (C) The levels of IL-18 in serum and colon; (D) The levels of TGF-β in serum and colon; (E) The levels of IL-10 in serum and colon; (F) The activity of MPO in serum. All data are presented as the mean ± S.E.M (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ (CON: the control group; LD: *L. casei* ATCC 393 + Dextran sodium sulfate; VD: Vasoactive intestinal peptides + Dextran sodium sulfate; LVD: *L. casei* ATCC 393 + Vasoactive intestinal peptides + Dextran sodium sulfate).

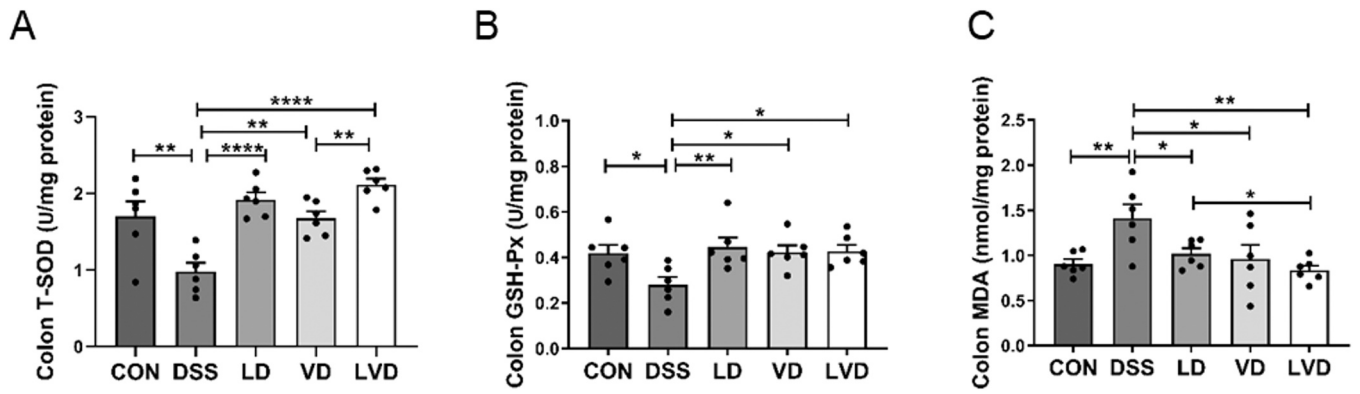

Fig. 5. The effect of *L. casei* ATCC 393 combined with VIP on the antioxidant capacity of UC mice induced by DSS. (A) The activity of T-SOD; (B) The activity of GSH-Px; (C) The content of MDA. All data are presented as the mean ± S.E.M (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ (CON: the control group; LD: *L. casei* ATCC 393 + Dextran sodium sulfate; VD: Vasoactive intestinal peptides + Dextran sodium sulfate; LVD: *L. casei* ATCC 393 + Vasoactive intestinal peptides + Dextran sodium sulfate).

with VIP can alleviate UC, the NF-κB signaling pathway was examined. The results showed that expression levels of the protein TLR4, MyD88, IKKα, p-NF-κB p65, p-IKBα and IL-1β in the DSS model group were significantly up-regulated, and the protein expression levels of NF-κB p65 and IKBα were significantly down-regulated (Fig. 6A–I). However, compared with DSS model group, the expression levels of TLR4, MyD88 and p-NF-κB p65 in *L. casei* ATCC 393 group, VIP group and *L. casei* ATCC 393 combined with VIP group were significantly down-regulated, while the expression levels of NF-κB p65 and IKBα protein were significantly up-regulated. In addition, the expression level of p-IKBα in *L. casei* ATCC 393 group and *L. casei* ATCC 393 combined with VIP group were significantly down-regulated. And the the expression level of IL-1β in VIP group and *L. casei* ATCC 393 combined with VIP group were significantly down-regulated. The expression levels of TLR4, MyD88, IKKα, p-NF-κB p65, p-IKBα and IL-1β in *L. casei* ATCC 393 combined with VIP group were lower than those in the other groups, while the expression levels of NF-κB p65 and IKBα were higher than those in the other groups. These results suggest that *L. casei* ATCC 393 combined with VIP may alleviate the inflammatory response of DSS-induced UC mice by inhibiting the activation of NF-κB pathway.

3.7. *L. casei ATCC 393 combined with VIP activates Nrf2-mediated signal pathway*

Nrf2 is an important transcription factor that regulates cellular oxidative stress, by regulating the expression of antioxidant proteins and thus preventing oxidative damage. As shown in Fig. 7A-D, compared with the control group, the expression levels of Nrf2, HO-1 and NQO1 were significantly down-regulated in the DSS-induced UC model group. However, compared with the DSS model group, the expression levels of Nrf2, HO-1 and NQO1 in *L. casei* ATCC 393 group, VIP group and *L. casei* ATCC 393 combined with VIP group were significantly up-regulated. Among them, the expression levels of Nrf2, HO-1 and NQO1 in *L. casei* ATCC 393 combined with VIP group were higher than those in the other groups. These results suggest that *L. casei* ATCC 393 combined with VIP may alleviate oxidative stress in DSS-induced UC mice by activating the Nrf2 pathway.

3.8. *L. casei ATCC 393 combined with VIP regulates the composition and diversity of colon microbiota in UC mice*

To investigate the relationship between UC and gut microbiota, we performed 16 S rRNA gene sequencing on the colonic contents of mice. As shown in Fig. 8A, there are 345 OTUs in the colon contents of each experimental group. Compared with control group, the ACE, Chao1 and Shannon indices of DSS model group were significantly decreased, while the α-diversity of gut microbiota in the other three groups has no significant difference (Fig. 8B–D). Compared with DSS-induced UC model group, the Simpson index of the VIP group increased significantly (Fig. 8E). In addition, the PCoA analysis showed that the diversity and composition of the gut microbiota in control group were significantly different from the other four groups (Fig. 8F). At the phylum level (Fig. 8G), compared with control group, DSS treatment significantly increased the abundance of Cyanobacteria and Proteobacteria and significantly decreased the abundance of Actinobacteriota and Firmicutes. Compared with DSS-induced UC model group, *L. casei* ATCC 393 combined with VIP group significantly increased the abundance of Firmicutes (Fig. 8H–K). At the genus level, compared with control group, DSS treatment significantly increased the abundance of *Bacteroides* and decreased the abundance of *Muribaculaceae* and *Turicibacter*. However, compared with DSS-induced UC model group, *L. casei* ATCC 393 combined with VIP significantly decreased the abundance of *Bacteroides* and significantly increased the abundance of *Turicibacter* (Fig. 9A–D).

3.9. *Correlation analysis of gut microbiota with biochemical indicators*

The spearman correlation heatmap of gut microbiota and biochemical indicators related to intestinal barrier dysfunction in UC mice was established to determine the potential correlation between DSS-induced changes in gut microbiota and biochemical indicators related to intestinal barrier dysfunction. As shown in Fig. 9E and Fig. S1, *Bacteroides* and *Escherichia-Shigella* are negatively correlated with IL-10 and TGF-β, while *Lactobacillus* was positively correlated with IL-10 and TGF-β. *Enterobacter* was negatively correlated with pro-inflammatory cytokines IL-18 and TNF-α, while *Turicibacter* was negatively correlated with IL-18 and positively correlated with TGF-β. These results showed that the increased abundance of *Lactobacillus*, *Enterobacter* and *Turicibacter* were helpful to alleviate the inflammation. *Enterobacter* and *Turicibacter* were positively correlated with the activity of T-SOD, while *Escherichia-Shigella* was negatively correlated with the activity of T-SOD. There was a significant negative correlation between the abundance of *Escherichia-Shigella* and the level of sIgA. There is a significant positive correlation between the abundance of *Escherichia-Shigella* and the level of MPO, and a significant negative correlation between the abundance of *Enterobacter* and the level of MPO.

4. Discussion

With the deepening of research, many evidences showed that inflammation, oxidative damage, impaired intestinal barrier function and gut microbiota dysbiosis played an important role in the etiology of UC [32]. UC involves the colon and colonic mucosa, which have a wide

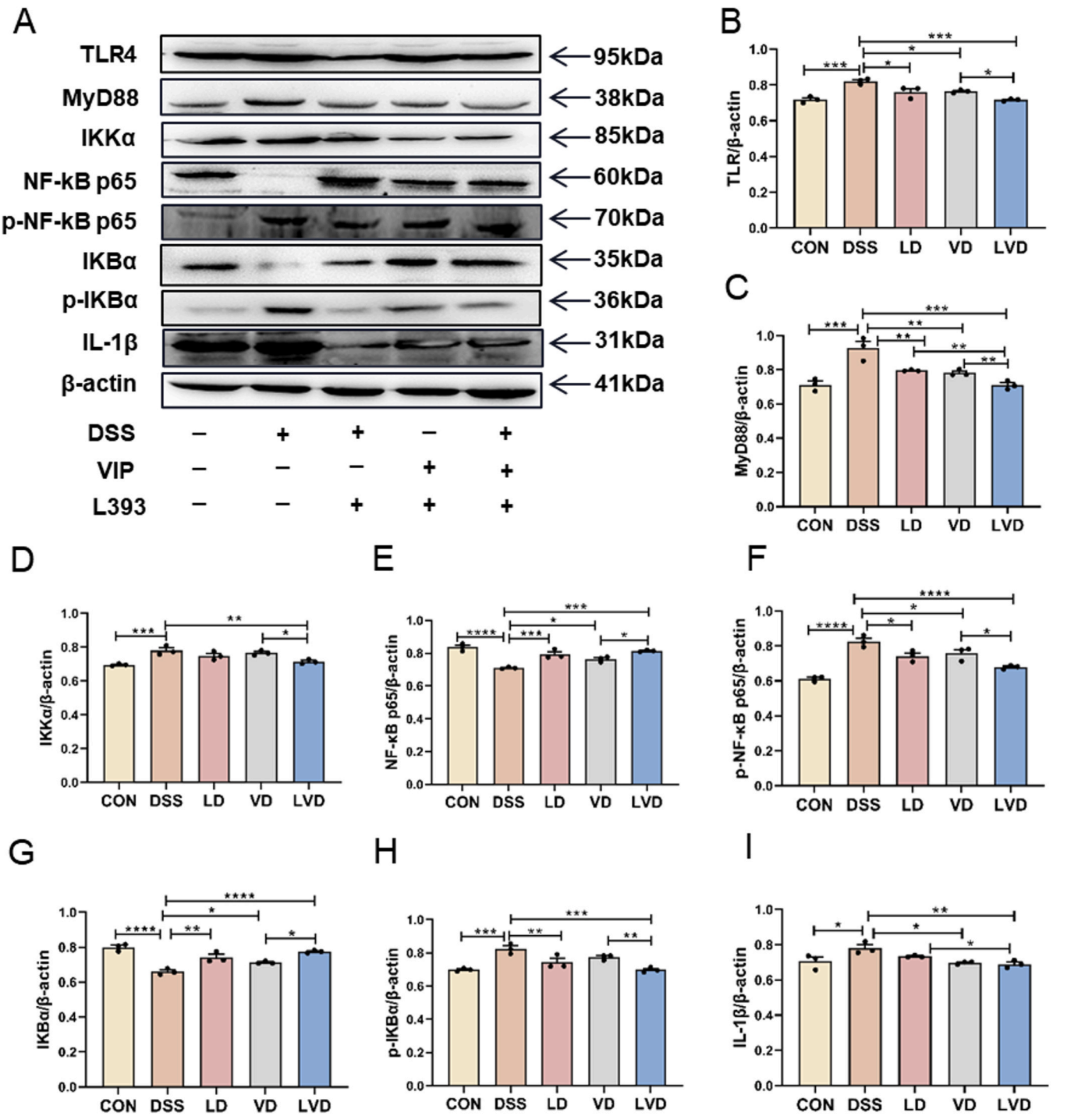

Fig. 6. The effect of *L. casei* ATCC 393 combined with VIP on NF-κB-mediated signaling pathway. (A) The expression levels of NF-κB-mediated signaling pathway-related proteins (TLR4, MyD88, IKKα, p-NF-κB p65, NF-κB p65, IKBα, p-IKBα and IL-1β) detected by Western blot analysis; (B-I) Quantitative analysis of the protein expression levels of TLR4, MyD88, IKKα, p-NF-κB p65, NF-κB p65, IKBα, p-IKBα and IL-1β. All data are presented as the mean ± S.E.M (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ (CON: the control group; LD: *L. casei* ATCC 393 + Dextran sodium sulfate; VD: Vasoactive intestinal peptides + Dextran sodium sulfate; LVD: *L. casei* ATCC 393 + Vasoactive intestinal peptides + Dextran sodium sulfate).

range of lesions, easy recurrence, poor therapeutic effect and high possibility of canceration. It has been listed as one of the contemporary refractory diseases by the World Health Organization. Studies have shown that DSS-induced UC in mice mainly affects the distal colon, with severe ulcer and recess loss, and the damage extends to the muscularis mucosa in mice [33]. In this study, the mice in the DSS-induced UC model group showed obvious weight loss, diarrhea, shortened colon, inflammatory infiltration and colonic recess atrophy. A study reported that the gut microbiota was likely to be a contributing factor to UC. Related clinical trials had similarly confirmed this idea that probiotics can alleviate UC by modulating the composition of the gut microbiota [34].

Probiotics are beneficial to the recovery of gut microbiota imbalance, intestinal immune response imbalance and the improvement of UC. It is reported that members of lactic acid bacteria have therapeutic properties, such as improving normal microbiome, reducing inflammation and

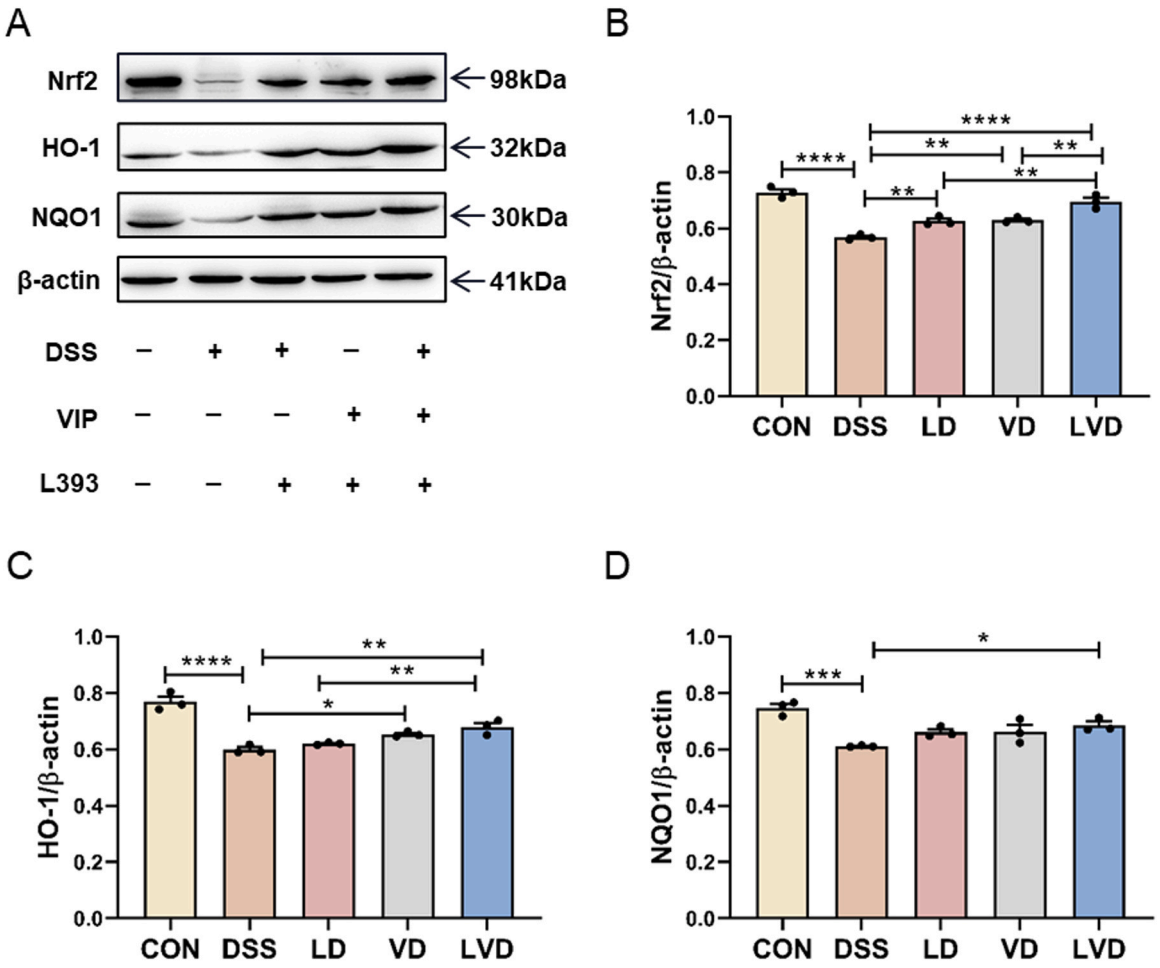

Fig. 7. The effect of *L. casei* ATCC 393 combined with VIP on Nfr2-mediated signaling pathway. (A) The expression levels of Nfr2-mediated signaling pathway-related proteins (Nrf2, HO-1 and NQO1) detected by Western blot analysis; (B-D) Quantitative analysis of Nrf2, HO-1 and NQO1 expression levels. All data are presented as the mean ± S.E.M (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ (CON: the control group; LD: *L. casei* ATCC 393 + Dextran sodium sulfate; VD: Vasoactive intestinal peptides + Dextran sodium sulfate; LVD: *L. casei* ATCC 393 + Vasoactive intestinal peptides + Dextran sodium sulfate).

stabilizing intestinal mucosal barrier, such as *Lactobacillus* and *Bifidobacterium*, can prevent and treat UC [35]. Probiotics can play some beneficial roles, such as anti-cancer, antioxidant and relieving UC [36]. At present, probiotics are very important in supporting the treatment of gastrointestinal diseases and autoimmune diseases [37]. A study reported that probiotics can change the inflammatory response by stimulating the production of cytokines. Probiotics can also enhance the intestinal barrier function and promote immune protection, thus improving the survival rate of intestinal epithelial cells [38,39]. In this study, *L. casei* ATCC 393 group stimulated the secretion of anti-inflammatory cytokines and inhibited the secretion of pro-inflammatory cytokines, thus alleviating the inflammation of UC. It has been proved that *Lactobacillus acidophilus* is widely used as a probiotic supplement in functional foods because of its beneficial regulatory effects on the host, such as immunomodulatory, anti-inflammatory and antioxidant activities [40]. In addition, some studies have proved that *Lactobacillus* have antioxidant capacity, and several studies have demonstrated that the components of *Lactobacillus* and *Bifidobacterium* can alleviate the damage caused by oxidative stress, example for UC [41]. In this study, we found that *L. casei* ATCC 393 effectively enhanced the antioxidant capacity of DSS-induced UC model mice. *Lactobacillus casei* has become one of the most widely used probiotics in food and dairy products. Milk fermented by *Lactobacillus casei* can improve the symptoms of patients with acute gastroenteritis [42]. Studies showed that the synergistic effect of *Lactobacillus casei* BL23 and milk can protect the integrity of intestinal epithelial barrier and reduce the damage caused by UC [43].

In recent years, many beneficial mechanisms have been found, including regulating gut microbiota, enhancing intestinal barrier function, protecting intestinal epithelium from pathogen invasion, and enhancing immune function. In this study, oral administration of *L. casei* ATCC 393 effectively alleviated DSS-induced intestinal barrier injury in mice. It is reported that oral administration of *Lactobacillus casei* can reverse the histological changes caused by TNBS, thus improving UC in mice [44]. However, the specific mechanism of anti-inflammatory and antioxidant effects of *Lactobacillus casei* remains to be determined. In this study, we found that *L. casei* ATCC 393 inhibits NF-κB signal pathway and activates Nrf2-mediated signal pathway. The activation of TLR4 can up-regulate the expression of downstream signal molecules MyD88, IKKα, p-NF-κB p65, p-IKBα and IL-1β, and down-regulate the expression of NF-κB p65 and IKBα. However, Nrf2 transcription factor was activated to up-regulate the expression levels of downstream transcription factors HO-1 and NQO1. A study also showed that the mechanism of action in treating UC disease is related to both NF-κB and Nrf2 signaling pathways. They found that the Maresin 1 plays a protective role in DSS-induced UC by activating Nrf2 signaling pathway and inhibiting TLR4/NF-κB signal pathway [45]. There is interaction between Nrf2 and NF-κB. The increased oxidative stress caused by the lack of Nrf2 will lead to NF-κB mediated cytokine expression increases, so NF-κB is more easily activated in an oxidizing environment. A study showed that potential crosstalk between Nrf2 and NF-κB exists. The activation of Nrf2 antioxidant signal transduction will weaken inflammatory signal

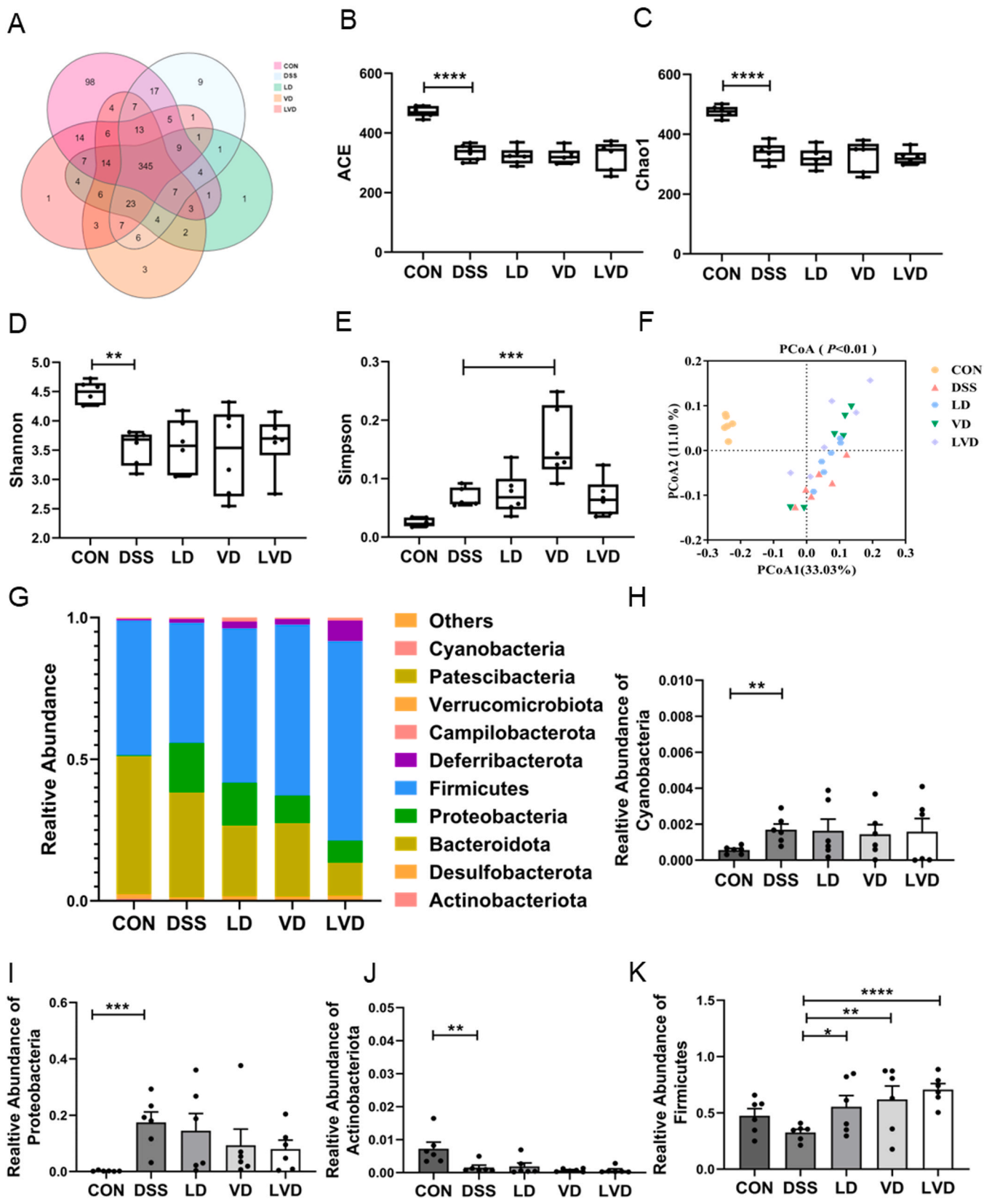

Fig. 8. The effect of *L. casei* ATCC 393 combined with VIP on the gut microbiota of UC mice induced by DSS. (A) Venn diagram; (B) The α-diversity analysis by AEC; (C) The results of α-diversity analysis by chao1; (D) The α-diversity analysis by Shannon; (E) The α-diversity analysis by Simpson; (G) The relative abundance composition of gut microbiota at the phylum level (n = 6); (H) Quantitative analysis of Cyanobacteria; (I) Quantitative analysis of Proteobacteria; (J) Quantitative analysis of Actinobacteriota; (K) Quantitative analysis of Firmicutes. All data are presented as the mean ± S.E.M (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ (CON: the control group; LD: *L. casei* ATCC 393 + Dextran sodium sulfate; VD: Vasoactive intestinal peptides + Dextran sodium sulfate; LVD: *L. casei* ATCC 393 + Vasoactive intestinal peptides + Dextran sodium sulfate).

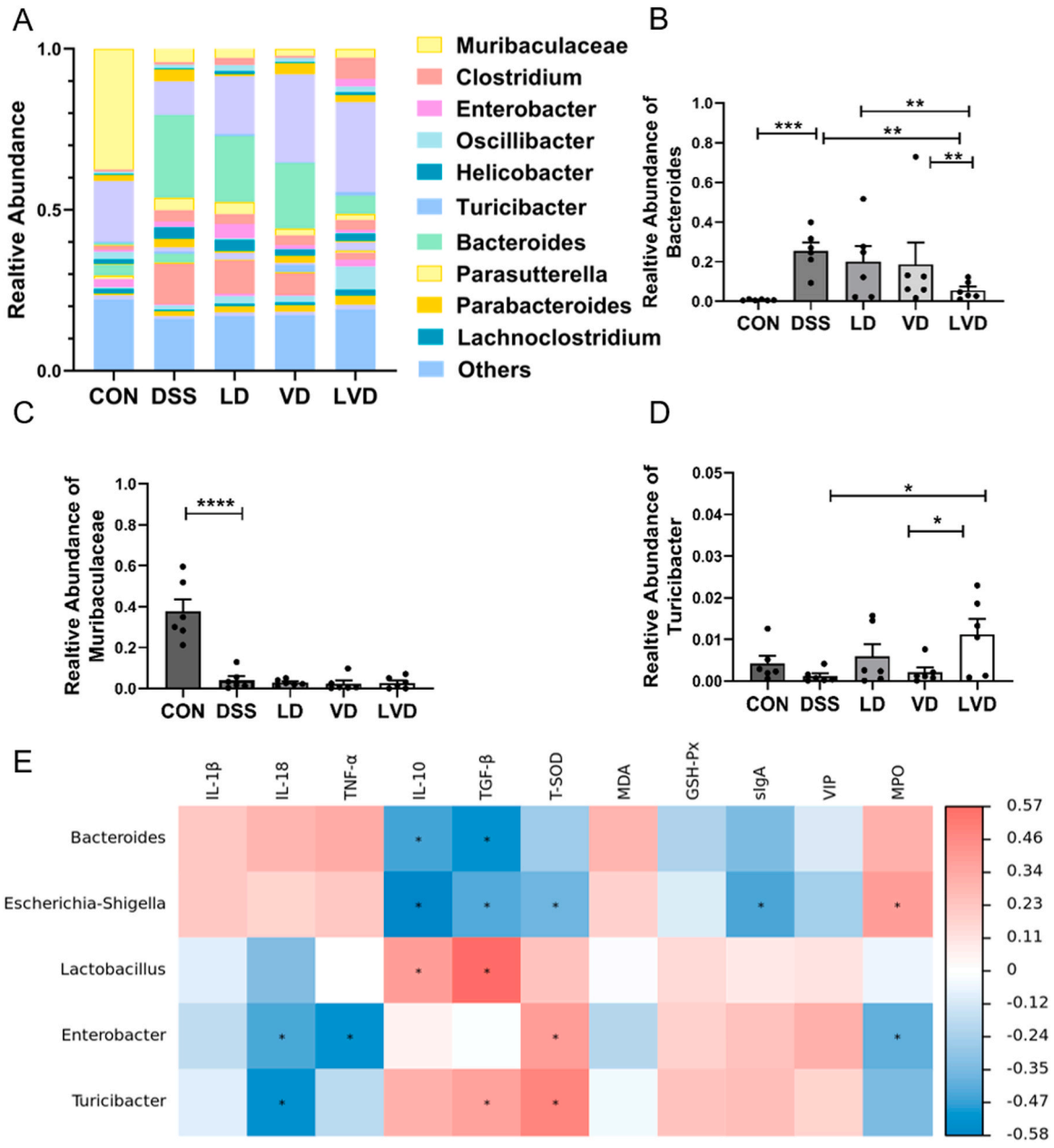

Fig. 9. The correlation of gut microbiota with biochemical indicators. (A) The relative abundance of gut microbiota at the genus level (n = 6); (B) Quantitative analysis of *Bacteroides* abundance; (C) Quantitative analysis of *Muribaculaceae* abundance; (D) Quantitative analysis of *Turicibacter* abundance; (E) Heatmap of Spearman's correlation between the abundance of gut microbiota and biochemical indexes related to UC induced by DSS. All data are presented as the mean ± S.E.M (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ (CON: the control group; LD: *L. casei* ATCC 393 + Dextran sodium sulfate; VD: Vasoactive intestinal peptides + Dextran sodium sulfate; LVD: *L. casei* ATCC 393 + Vasoactive intestinal peptides + Dextran sodium sulfate).

pathway of NF-κB. Olmesartan administration by activating Nrf2 to inhibiting NF-κB signal transduction pathway, thereby effectively treating UC [46].

Gut microbiota imbalance has been proved to be directly or indirectly related to various diseases, from inflammation and metabolic diseases to nervous system diseases and cancers [47]. Many studies have showed that understanding the complex relationship between intestinal immune system and microbiome will help to further clarify the pathogenesis of UC and develop treatment methods [48]. The dysbiosis of gut microbiota can reduce the diversity of gut microbiota, thus damaging the intestinal mucosal homeostasis. Restoring microecological disorder to maintain the diversity of gut microbiota can provide a new idea for the treatment of UC patients [49]. Furthermore, it is reported that the composition of gut microbiota in patients with UC will change, including the decrease of microbial diversity and the increase of facultative anaerobic bacteria, such as the decrease of the abundance of Firmicutes, the disappearance of *Clostridium*, and the increase of the abundance of *Enterobacter* [50]. In this study, the abundances of Firmicutes, *Bacteroides* and *Clostridium* were also observed to decrease, but the abundance of *Enterobacter* increased in DSS-induced UC model mice. A study demonstrated that gut microbiota plays an important role in mucosal immune response in intestinal diseases and inflammatory reactions. UC can be treated by regulating gut microbiota and increasing the number of probiotics [51]. However, in this study, the *L. casei* ATCC 393 group, the VIP group, and the *L. casei* ATCC 393 combined with VIP group did not reflect significant effects such as enriching the diversity of mice gut microbiota and changing the composition of mice gut microbiota. This indicates that the gut microbiota does not play a major role in *L. casei*

ATCC 393 combined with VIP group to treatment of DSS-induced UC. In addition, this study further found that the abundances of *Bacteroides*, *Escherichia-Shigella*, *Lactobacillus*, *Enterobacter* and *Turicibactor* are closely associated with the inflammatory cytokines and oxidation indicators.

VIP can regulate intestinal vasoconstriction and secretion. It has been showed that VIP plays a vital role in the development and maintenance of the integrity of colonic epithelium and mucus barrier [52]. Previous research had proved that VIP can prevent epithelial barrier destruction and UC induced by bacterial pathogens [53]. In this study, intraperitoneal injection of VIP can increase the content of sIgA and up-regulate the expression levels of occludin and claudin 1, and effectively maintain the integrity of intestinal epithelium and mucus barrier. In particular, VIP is a multifunctional neuropeptide produced by lymphocytes and nerve cells, which has a wide range of immune functions [54]. VIP has been known to have many functions on intestinal tract. Studies have shown that VIP in colon mucosa of UC patients is significantly decreased, and the decrease of VIP content in mucosa may affect local inflammatory response and disease activities [55]. In the past decade, VIP has been clearly identified as an effective anti-inflammatory cytokine, which works by regulating the production of anti-inflammatory mediators and pro-inflammatory mediators [56]. Intravenous injection of VIP can significantly improve the clinical severity of UC, eliminate symptoms such as weight loss, diarrhea and bloody stool, and greatly improve the survival rate [57]. Therefore, VIP is considered as a promising alternative treatment for acute and chronic inflammation [58]. In this study, we found that intraperitoneal injection of VIP can effectively reduce the severity of DSS-induced UC. In UC mice, some authors have shown that intraperitoneal (ip)VIP has protective effect on mucosal inflammation by inhibiting pro-inflammatory cytokines and down-regulating toll-like receptors 2 and 4 [23]. Our research is consistent with the previous report. The expression levels of IL-1β, IL-18 and TNF-α in mice injected with VIP by intraperitoneal injection was inhibited, and the inflammatory pathway protein TLR4 was down-regulated, which effectively alleviated the inflammation degree of DSS-induced UC.

In this study, *L. casei* ATCC 393 can alleviate UC by reducing inflammatory reaction, enhancing antioxidant capacity, inhibiting NF-κB signal pathway, and activating Nrf2 signal pathway. VIP also has a certain therapeutic effect on UC, which can regulate the secretion of inflammatory cytokines and oxidation markers, inhibit NF-κB signal pathway, activate Nrf2 signal pathway, and further protect the intestinal barrier from damage. Although both of them can relieve UC to varying degrees, their combined effects are more effective for the treatment of UC. It can be seen from the experimental results of the three groups of protection groups that, by measuring the length of the mouse colon, the recovery of the mice colon shortening is most obvious in the *L. casei* ATCC 393 combined with VIP group. H&E showed that the phenomenon of crypt atrophy in *L. casei* ATCC 393 combined with VIP group improved most obviously. In addition, *L. casei* ATCC 393 combined with VIP group had the lowest FITC-dextran content and the most obvious up-regulation of tight junction protein expression, indicating that *L. casei* ATCC 393 combined with VIP group had the most prominent role in restoring intestinal permeability. However, *L. casei* ATCC 393 combined with VIP group had the highest expression of anti-inflammatory cytokines, the lowest expression of proinflammatory cytokines, and the highest content of antioxidant markers. Inflammation and oxidative stress are related to the development of UC. NF-κB signaling pathway is related to inflammation, and Nrf2 signaling pathway is related to oxidative stress. Previous research found that NF-κB signal dysfunction is closely related to the pathogenesis and progress of UC [59]. Targeting TLR4/NF-κB signal path is an effective way to relieve UC [60,61]. Furthermore, it is reported that Nrf2/HO-1 signaling pathway is one of the key pathways to regulate various antioxidant and inflammatory mechanisms and activating Nrf2 pathway can effectively improve DSS-induced UC in mice [62]. Nrf2 signalling and NF-κB signalling interact. Previous studies have demonstrated that the activation of Nrf2 signalling has anti-inflammatory effect and can inhibit NF-κB signalling [63,64]. Additionally, a study found that myristicin treats oxidative stress and inflammation caused by UC *in vivo* increasing the expression of Nrf2 and inhibiting the activation of NF-κB [65]. In this study found that both *L. casei* ATCC 393 and VIP can alleviate UC by inhibiting NF-κB signal pathway and activating Nrf2 signal pathway. It is worth noting that *L. casei* ATCC 393 combined with VIP group is more effective in inhibiting NF-κB signal pathway and activating Nrf2 signal pathway. *L. casei* ATCC 393 combined with VIP can up-regulate the expression of Nrf2 by activating Nrf2 signal pathway and activate its downstream oxidation factors HO-1 and NQO1 to up-regulate their expression, so as to regulating endogenous oxidative stress. Oxidative stress in the organism is closely related to the occurrence of inflammation. Therefore, *L. casei* ATCC 393 combined with VIP can inhibit NF-κB signal pathway and down-regulate downstream factors TLR4, MyD88, IKKα, p-NF-κB p65, p-IKBα and IL-1β, up-regulate NF-κB p65 and IKBα to regulate inflammation in the organism. Consequently, NF-κB and Nrf2 signal pathway is the key mechanism for treating UC. *L. casei* ATCC 393 combined with VIP can alleviate a series of adverse reactions such as intestinal barrier damage, inflammation and oxidative stress caused by DSS-induced UC through this pathway.

In conclusion, *L. casei* ATCC 393, VIP and *L. casei* ATCC 393 combined with VIP group can effectively alleviate DSS-induced UC in mice by maintaining the intestinal barrier integrity, enhancing the antioxidant capacity and regulating inflammatory response. The mechanism is closely associated with NF-κB and Nrf2 signaling pathways. Noteworthy, *L. casei* ATCC 393 combined with VIP was more effective than *L. casei* ATCC 393 or VIP alone in protecting against DSS-induced UC. The exact effects and mechanism of probiotics combined with VIP need further investigation. Overall, this study provides a novel strategy to prevent and treat UC.

Ethics approval and consent to participate

The animal used in this study was approved by the Institutional Animal Care and Use Committee of the Northwestern Polytechnical University (Permit Number: 202201097) and conducted in accordance with the National Institutes of Health guidelines for the care and use of experimental animals.

CRediT authorship contribution statement

Lixu Zhu: designed the experiments, performed animal studies, performed molecular biology experiments, deal with experimental data and prepared the manuscript. **Lei Qiao:** designed the experiments, performed animal studies, deal with experimental data and modify the manuscript. **Xina Dou** and **Xiaofan Song:** performed animal studies and modify the manuscript. **Jiajing Chang** and **Xiaonan Zeng:** performed animal studies. **Chunlan Xu:** designed the experiments, modify the manuscript and were primarily responsible for the final content. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.115033.

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